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KINETICS OF β-[^{14}C]CAROTENE IN A HUMAN SUBJECT USING ACCELERATOR MASS SPECTROMETRY

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β-Carotene is a tetraterpenoid distributed widely throughout the plant kingdom. It is a member of a group of pigments referred to as carotenoids that have the distinction of serving as metabolic precursors to vitamin A in humans and many animals [1,2]. We used Accelerator Mass Spectrometry (AMS) [3] to determine the metabolic behavior of a physiologic oral dose of β-[^{14}C]carotene (200 nanoCuries; 0.57 μmol) in a healthy human subject. Serial blood specimens were collected for 210-d and complete urine and feces were collected for 17 and 10-d, respectively. Balance data indicated that the dose was 42% bioavailable. The absorbed β-carotene was lost slowly via urine in accord with the slow body turnover of β-carotene and vitamin A [4]. HPLC fractionation of plasma taken at early time points (0-24-h) showed the label was distributed between β-carotene and retinyl esters (vitamin A) derived from intestinal metabolism.

MATERIALS AND METHODS: β-[^{14}C]carotene was prepared by growing spinach in an atmospherically sealed labeling chamber pulsed with $^{14}CO_2$ [5,6,7]. The β-carotene was extracted and final radiometric and chemical purity was determined to be greater than 98% by RP-HPLC (S.A. 0.35 mCi/mmol). The β-carotene was dispersed in olive oil and a 200 nanoCi aliquot was transferred to a gelatin capsule equivalent to 0.57 μmol (0.306 mg) for ingestion. Pre-dose urine and feces were collected a day before dosing. Pre-dose blood was drawn just before the dosing. Blood samples were drawn every 30-min for 10-h postadministration and at longer intervals thereafter for 210-d. Complete 6-h urine collections (4) were made during the first 24-h period. Complete 24-h collections were continued from day 2 through day 17. Complete fecal collections were made through day 10. Plasma was separated from blood cells by centrifugation. Fecal collections were dispersed with 0.5 M KOH; aliquots were removed for AMS and carbon analysis. Plasma samples were analyzed neat and following HPLC separation of major metabolites. Samples were combusted and reduced to graphite [8] for AMS measurement [9,10]. Natural ^{14}C was subtracted from measured isotope ratios.

RESULTS AND DISCUSSION: AMS measurements were done to ±3% precision (instrument precision) as measured by the standard deviation of 3 or more measurements of the ^{14}C concentration [3,10]. The assay precision was determined from the amount of scatter in the results obtained from repeat analyses of a homogenous sample and is the primary determinant of detection limit. Accordingly, absolute sensitivity per sample

based on double the uncertainty in the assay precision was 18, 32, and 17 amol β-[^{14}C]carotene per plasma, urine, and fecal specimen, repectively. Collected HPLC fractions had detection limits of 3 amol β-[^{14}C]carotene/fraction.

The concentrations versus time course of the label in plasma analyzed neat (A) and following HPLC fractionation (B) are shown in figure 1. The neat concentration profile displayed a 5.5 hour delay before appearance of label (A). The 5.5 hour lag is in accord with the relatively slow absorption kinetics of lipophilic compounds via the lymphatic system. Slow gastric emptying may also contribute to this lag. The initial peak occuring between 5.5 and 10 hours reflects the appearance of label into circulation aboard short-lived chylomicron particles of intestinal origin. These particles and their lipophilic contents are rapidly removed from circulation by the liver ($t_{1/2}$ ~11 m) which explains the bell-shaped (guassian) profile. Subsequent resecretion of labeled compounds from the liver aboard longer-lived lipoproteins and specific carrier proteins results in a broad secondary peak of higher concentration. Sufficient signal was present for determinations

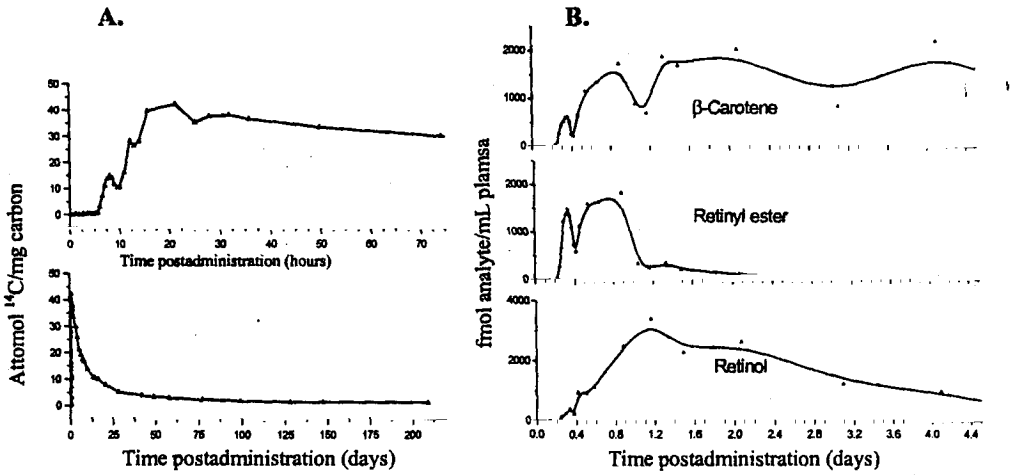

Fig. 1. Profiles of concentration versus time course of label in plasma analyzed neat (A) and following HPLC fraction of select metabolites (B).

209 days postadministration (A, bottom panel). Fractionation of labeled metabolites by HPLC followed by AMS analysis revealed the concentration time course of β-carotene and its metabolites, retinyl esters and retinol (B). It is apparent that the initial absorptive peak starting at 5.5 h is similar in shape to that of the neat profile and is a composite of the β-carotene and retinyl ester concentration profiles, suggesting these compounds share a common absorption process. Plasma retinol rises linearly (bottom panel) for ~ 24-h

postdosing reflecting the postabsorptive hepatic biotransformation of retinyl esters into retinol and the subsequent release into circulation with specific retinol binding proteins.

Cumulative urinary and fecal output as a percentage of dose were determined. Absolute bioavailability determinations using this approach are quantitative and facile. Based upon the recovery of 58% of the dose after 48-h in the feces, dose bioavailability was 42%. Urinary excretion rates were low: 5% of the administered dose and 2% of the absorbed dose was excreted in the urine after 17-d. The slow elimination rate is attributed to the low aqueous solubility of the compounds and long biological half-lives (estimated from the final elimination portions of the concentration vs. time curves, β-carotene and retinol have half-lives of ~40 and 210-d, respectively).

CONCLUSION: AMS is an excellent bioanalytical tool for in vivo human nutrient studies using long-lived radioisotopes such as ^{14}C [11]. AMS sensitivity (attomolar) enables radiocarbon tracer studies to be conducted at low microSievert radiation exposures using physiologic-sized doses. The methodology will facilitate determination of fundamental kinetic parameters for nutrients and bioactive phytochemicals.

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